Interplay between productive traits, the social rank, and the cow's stability in the order of entrance to the milking parlour

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Standards

Animal care and procedures were carried out according to the guidelines of the Animal Care and Use Committee of the Pontificia Universidad Católica de Chile (ID No. 150908002).

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Abstract

The aim of this study was to investigate whether social rank and stability in the order of entrance to the milking parlour are associated with production traits. The study was conducted on a dairy farm where cows (n = 215) were managed in three groups according to lactation stage (Group 1: 78 cows 0-100 DIM; Group 2: 65 cows 101-200 DIM; and Group 3: 72 cows >200 DIM). Social rank (SR) was calculated from observations made from agonistic behaviour performed at the water troughs and feed bunks of each pen (n = 3). The animals were classified in 3 levels of dominance based on at least 5 clear interactions, resulting in: 61 dominant, 75 intermediate and 69 subordinate cows based on SR. Stability in the order of entry was estimated as the standard deviation of the entry position. SR was weakly correlated to milk yield, urea and protein content in milk. The results showed that stable cows had higher milk production and entered the milking parlour after the non-stable animals. Stability in the order of entry to the milking parlour was not affected by social rank. Overall, the use of milking facilities appears to be associated with production traits rather than social rank.

Keywords: Social hierarchy, animal welfare, dairy, milking order.

Introduction

Dairy cows form a social hierarchy through dominance establishment and this is associated with higher-ranking individuals having supremacy in the use of resources (Phillips and Rind, 2002). In a dairy herd, the social rank is related to factors such as age and animal size that can be measured as body condition score and live weight (Sottysiak and Nogalski, 2010). These factors are associated with physical strength and the competition for space that becomes a driver for aggressiveness of cows in confined spaces (Galindo and Broom, 2000). Aggressive social interactions create stress conditions between dominant and subordinate cows, which may affect milk production, especially for the subordinate cows (Chebel et al., 2016)

Dairy cows enter the milking area in a specific order and their behaviour is often expressed in a relatively consistent pattern (Paranhos da Costa & Broom 2001; Polikarpus et al., 2015). Consequently, preference for one side of the milking parlour seems to be a stable characteristic of a dairy cow (Hopster & van der Werf 1998; Grasso et al., 2007). Social rank (Melin et al. 2006), health-related characteristics (Flower et al. 2006; Polikarpus et al. 2015) and productive-related characteristics (Górecki & Wójtowski 2004) can influence the efficient use of milking facilities. For example, social rank, social structure of the herd and individual characteristics, such as anxiety, fear and stress, can influence side preference (Prelle et al. 2004). In addition, cows with locomotion and udder health problems can have problems to reach milking facilities (Gleeson et al., 2007).

The knowledge about milking order in dairy systems may provide data that can be used for early detection of health problems and prevention of production and economic losses (Polikarpus et al., 2015). Many studies have pointed out different factors affecting the use of milking facilities but to our knowledge, the interplay between productive traits, the social rank, and the stability of cows in the order of entrance to the milking parlour remain unexplored. Thus, the aim of this study was to determine whether social rank and stability in the order of entrance to the waiting area and milking parlour were affected by productive traits. To address the study´s objective animals were initially classified by their social rank. We investigated the relationship between social rank and: (a) health related traits (locomotion score, body condition score and udder hygiene score); (b) stability in the order of entrance to the waiting area and milking parlour; and (c) and milk production.

Materials and Methods

Animals, housing and management

Animal care and procedures were carried out according to the guidelines of the Animal Care and Use Committee of the Pontificia Universidad Católica de Chile (project ID No. 150908002). The study was conducted in a commercial dairy farm located in Pirque, Chile (33°38′28″S, 70°34′27″W). Lactating cows (n = 205; Holstein × Montbeliarde) were managed in three groups according to lactation stage (Group 1: 63 cows 0-100 DIM; Group 2: 77 cows 100-201 DIM; and Group 3: 65 cows >200 DIM). Animals were housed in dry lots (n=3; 80 × 80 m) with shade and had continuous access to water. Each pen had a rectangular-shape concrete water trough (3.5 × 1.5 m) and feed bunks were located in one side of the pens (approximately 60 linear meters per pen). Cows were fed three times per day (08:00, 10:00 and 17:00 h). Animals were milked in a 2 × 6 parallel milking parlour equipped with DELPRO™ farm manager system (DeLaval, Sweden), three times per day (03:30, 12:00 and 18:00 h). The walking distance from the pens to the milking parlour varied from 50 to 100m, depending on the group's location. Milk yield was recorded daily and analysed as total means. Body weights were individually measured after the first milking using a livestock chute with a weighing bar system. Body weights were recorded every 6 days and means were analysed. Milk components were recorded every 21 days (milk components were analysed by using an infrared analyser: Milko-Scan CombiFoss 6000; Foss Electric, Hillerød, Denmark) and means were analyzed.

Behavioural observations and social rank determination

During 42 days, agonistic interactions (agonistic encounters included bunting, pushing, threatening, avoiding and fighting) were registered to calculate the social rank index of each animal and its subsequent correlation analysis with productive traits (milk production, somatic cell count, locomotion score, body condition score and udder hygiene score). In addition, the sequence of entrance to the waiting area and the milking parlour was used to define two indices of individual stability.

Behavioural observations were undertaken using a video recording system (SONY, YC-231G). Numbered coloured collars were used to facilitate animal identification. Agonistic interactions were registered through direct observation at the water trough after two milking times (12:00 and 18:00 h) during two consecutive days. Water trough access was denied from the moment the group left the pen to be milked until 30 minutes after the last cow entered to the pen (approximately one hour and 40 minutes without having access to water troughs in total). Each group was observed during five consecutive days for 30 hours. At the feed bunks, during two consecutive days, agonistic interactions were registered through direct observation two times per day (08:00 and 17:00 h). Each group was observed for 1 hour and 30 minutes by the same observer.

A social rank (SR) was calculated for each cow within each group from all observations (from water troughs and feed bunks). This SR was calculated based on methodology described by Galindo et al. (2000) and Hohenbrink and Meinecke-Tillmann (2012): Social rank = interactions won /(won + lost interactions). Animals showing a score of 0.0 to 0.4 were classified as subordinates. Individuals with a score from >0.4 to 0.6 were classified as intermediate. Cows with a score from >0.6 to 1.0 were classified as dominant.

Health and productive related characteristics

The locomotion score (LS), body condition score (BCS) and udder hygiene score (UHS) were considerate as health and productive related characteristics. After behavioural observations, these characteristics were recorded at the end of the midday milking (12:00 h) during six consecutive days. The LS considered five individual aspects (spine curvature, tracking, speed, head carriage and abduction/adduction) of locomotion in a five-point scale as reported by O'Callaghan et al. (2003) and observations were done for once daily for 6 consecutive days. The BCS was based on a five-point scale where 1 = emaciated to 5 = overly fat (Wildman et al. 1982). A four-point score was used to measure the UHS based on Ruegg (2006) where 1= completely free of dirt or has very little dirt; 2= slightly dirty; 3 = mostly covered in dirt; and 4= completely covered, caked on dirt. The same person who was trained for those measurements carried out scorings for locomotion, body condition and udder hygiene.

Milk production and stability in the order of entrance to the waiting area and milking parlour

An automated management information system (DELPRO™, DeLaval, Sweden) recorded the entrance time to the milking parlour, milking duration, milking unit and individual milk yields and associates them with the ear tag embedded identification from each cow. Before each milking, all cows from each group were taken together to a waiting area. During this period, cows were free to choose their position in relation to the entrance in the milking parlour without any intervention of the stockman. The waiting area (120 m^2) was in front of the milking parlour (Figure 1). Both sides of the milking parlour were identical and accessed through automatic gates. The gates were open as soon as a milking unit was empty.

The entrance order to the waiting area and the milking parlour were video-recorded (SONY, YC-231G). Nine records of entrance positions to the waiting area were obtained from each animal. For the entrance to the milking area 15 records of positions per animal were obtained. With these data, the individual stability of each animal was calculated as the standard deviation of its position records. With this information, 30% of the most stable cows and 30% of the less stable cows were selected to form two stability groups for production trait analyses. These groups (stable and non-stable) were compared for production characteristics such as milk production, urea, protein, fat and somatic cells in milk.

The sequence of entrance to the milking parlour was determined using the data obtained by the DelPro ™ program (DeLaval) routinely used in the dairy farm, where the starting time of milking and milking unit used by each cow was recorded. Based on the different numbers of animals from Groups 1, 2 and 3, the positions in the order of entrance to the waiting area and milking parlour were standardize from 1 to 9.

Statistical analysis

Correlations were analysed using Pearson (parametric data: social rank, milk composition and milk yield) or Spearman (non-parametric data: locomotion score, body condition score, udder hygiene and dominance level) tests. Additionally, Chi-square tests were performed to assess whether there was a dependency between Locomotion score (LS), body condition score (BCS) and udder hygiene score with social rank.

Productive characteristics, social rank, and order of entrance to the waiting area and to the milking parlour from stable and non-stable cows were analyzed by using ANOVA. The groups of cows (stable and non-stable) and their lactation stage were considered as fixed effects in the following model:

$$Y_{ijk} = \mu + \alpha_{i} + \beta_{k} + (\alpha\,\beta)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable; μ is the overall mean; α_i is the effect of lactation stage; β_k is the effect of stability group; and ε_{ijk} is the experimental error.

The homogeneity of variances was analysed with the Levene test. This was followed by the Student-Newman-Keuls multiple comparison of means procedure at $P < 0.05$ to determine differences between the different groups. Results were expresses as mean ± standard deviation. Probability of $P < 0.05$ was defined as significant. The SPSS statistical software for Windows was used (version 15.0.0; SPSS Inc., Chicago IL, USA). When statistical analyses were performed, the outliers identified were identified as the consequence of extraordinary events, such as health problems (i.e., clinical mastitis and abortion), and the need for some veterinary treatment. Therefore, as they did not represent an important segment of the study population, they were eliminated from the analyses.

Additionally, to assess whether there was an effect of position on the standard deviation of the position data, the cows were grouped into 9 groups according to the average position obtained during the observations, and the SD of their locations was calculated. ANOVA or Kruskal-Wallis test were performed with these data, after heteroscedasticity test of variances. When there was no heteroscedasticity of variance, the multiple comparison was performed using the Tukey's

Honest Significant Difference test, and otherwise, through the non-parametric test by Notched Box Plots.

Results

Out of the initial 215 cows in the herd, five cows were culled out due to health problems during the study period; one additional cow had an abortion and was discarded from the study due to the need for veterinary treatment. Of the remaining 209 cows, 4 were eliminated from the results database, because they presented outlier values in some of the variables analyzed. Table 1 shows the final production and health related characteristics for each group. Briefly, group 1 consisted of 77 animals, of which 27% were dominant, 38% were intermediate and 35% were subordinate. Group 2 (n = 63) had a distribution of 32% dominant animals, 29% of intermediate animals and 38% of subordinate animals. Group 3 (n = 65), where the animals in their last third of lactation had a distribution of 30% of dominant animals, 39% of intermediate animals and 31% of subordinate animals. The social rank evaluation from all animals (n = 205) resulted in 61 dominant animals, 75 intermediate animals and 69 subordinate animals. The agonistic interactions during water consumption represented less than 20% of the total interactions (Table 2).

There was a significant correlation between the SR and parity, which means that multiparous cows tend to be dominant individuals in the herd (Table 3). A weak correlation between parity and locomotion score (r = 0.464, $P < 0.001$) was found, which in a way indicates that animals with more parities have higher locomotion scores. Body condition score had a tendency to be weakly correlated with the dominant animals, where dominant animals had better scores than the subordinate animals (r = 0.126, $P = 0.095$). Table 4 shows the distribution of locomotion score, body condition score and udder hygiene score of cows within social ranks. Regardless of social rank in the herd, 88% of the cows had a locomotion score of 2, 86% of the cows had a body condition score between 2.75 and 3.25 and 78% of the cows had a udder hygiene score between 1 and 2. The Chi-square test showed that there was no dependency between locomotion score [Xi (205) 6, 3.94, $P = 0.689$], body condition score [Xi (205) 10, 14.3, $P = 0.155$] and udder hygiene score [Xi (205) 6, 4.3, $P = 0.634$] on social rank (Table 4).

The stability in the order of entrance to the waiting area had a significant effect on milk production, thus, stable cows had higher milk production than non-stable cows (Table 5). In relation to milk fat, stable cows presented a higher content at the waiting area and in the milking parlour, however, significant differences were only observed in the milking parlour. The stability in the order of entrance also affected the concentration of urea at the waiting area with higher contents detected in stable cows.

As shown in Figure 2, the standard deviation of the position of the cows was related to their average position, thus, at the waiting room, the ANOVA test was significant, and differences were observed between the standard deviations of each category. Thus, the Kruskal-Wallis test was used, which gave a value of $P = <0.001$. This test showed differences between categories 1, 2 and 9, which presented the smallest standard deviations, with the central groups from 4 to 7. At the milking parlour, the ANOVA test was not significant ($P = 0.068$) and, as in the waiting room, the standard deviation of the data presented heteroscedasticity, so the Kruskal-Wallis test was used, which was significant ($P = 0.004$), obtaining differences between the means of groups 1, 2 and 8 with group 4.

Discussion

Productive traits and social rank

In this study, the social rank was determined on agonistic interactions from each group that was observed during the first minutes when there was no access to water. These findings coincide with a study conducted by Kondo et al. (1984) who observed a rapid decrease in the frequency of agonistic encounters as time passes. Similarly, at the feed bunk, Menke et al. (2000) reported that agonistic behaviour decreases in a relative short period.

Normally, the hierarchy is expressed mainly at the feed bunks (Val-Laillet et al. 2008). In this study, cows had water *ad libitum* after the 1:40 min restriction imposed after milking and feed was offered three times a day. In this regard, feed availability was restricted creating more competition among animals, resulting in a greater number of agonistic interactions at the time of feeding (Proudfoot et al. 2009). The results of a study conducted by DeVries and Von Keyserlingk (2006), concluded that dairy cows experience a lower number of agonistic interactions when they are given more than the standard of 0.61 m of feeder/animal (Grant & Albright 2001). In the current study, animals had 0.8 m/animal. Previous studies showed that fresh cows, first calving heifers and cows recently integrated into a group are submissive cows; and larger and older cows in a group are often the most dominant (Guhl & Atkeson 1959; Grant & Albright 2001; Phillips & Rind 2002).

The correlations between locomotion scores and parity found in this study were weak, however they pointed at the fact that older animals are more prone to having foot problems (Galindo & Broom 2000). Hetticha et al. (2007) reported that the number of births and, therefore, the age, was a risk factor for the presentation of this pathology, where cows with three or more lactations presented 3.8 times more chances of suffering from this condition. This agrees with Sogstad et al. (2005), who found that cows with three or more lactations had higher risks of lameness than those with less than three lactations. In this study, animals that are more dominant had increased body conditions scores. This finding suggest that dominant animals have unrestricted access to different resources (i.e., feed, water, and bedding), while subordinate animals have restricted access to the feeding places, which could affect their feed intake (Dickson et al. 1970; Llonch et al., 2018) and, therefore, their body condition score. Overall, in this study, given the weak correlations and non-significant Chi-square values found between locomotion score, body condition score, udder hygiene score and social rank. These results therefore, should be interpreted with caution as this was based on observations from 205 animals, which was not sufficient to have a greater dispersion of scores.

There was a weak correlation between milk yield (mean milk yield for each cow) and dominance; dominant animals had higher performance than the subordinate animals ($r = 0.191$, $P <0.001$). In dry-lot pens, where feeding is not available *ad libitum*, animals unable to adapt to a competitive environment may be at a disadvantage in terms of the quantity and quality of the feed they have access to (Zobel et al. 2011), which could affect their consumption and, therefore, decrease their production. It is very important that the cows have good access to the diet and water throughout the day, to ensure the health, welfare, production and efficiency of the cow (Shabi et al. 2005). In farms where feed is limited, and competition is high, dominant cows eat more feed (Grant & Albright 2001) and drink more water (Teixeira et al. 2009) than subordinate cows.

There were weak correlations between social rank and body weight ($r = 0.276$, $P < 0.001$), parity ($r = 0.339$, $P < 0.001$) and milk yield ($r = 0.191$, $P < 0.001$). In this study, when social rank was determined at the water troughs and feed bunks, it was observed that dominant animals with larger body size, had unimpeded access to feed and water, while the subordinates were displaced and forced to move to other places without being able to consume feed. These results coincided with the study on a single herd carried out by Dickson et al. (1970), who suggested that live body weights and age were the factors most closely related to the position of the cow in the social hierarchy. In addition, Schein and Fohrman (1955) reported a high significant relationship between the range of dominance and age, and live body weight as well. On the other hand, the correlation between the social rank and body condition score and udder hygiene were low; the correlation coefficients were between 0.096 and 0.126 (Table 3). No significant correlation was observed between SR and locomotion scores nor somatic cell count (Table 3).

Stability in the order of entrance to the waiting area and milking parlour

At the waiting area, we observed a relationship ($P = 0.008$) between SR and milk yield, since stable cows (lower standard deviation) were those with higher milk yield, compared to non-stable cows (higher standard deviation) (Table 5). Furthermore, although stable cows entered into the waiting area 0.12 places after non-stable cows (Table 5) this is not expected to be biologically significant. According to (Grasso et al. 2007) the cows have a coherent order of entry to the waiting area, which is considered as a social characteristic of the animals belonging to a dairy system.

The milk urea contents in both groups are within the normal expected range (150 to 420 mg/L) (Westwood et al. 1998). Stable cows had higher contents of milk urea. The concentration of milk urea is related to the supply of nitrogen in the diet, specifically the balance between degradable proteins and energy in the rumen. A high nitrogen intake or an energy deficiency produce an excess of ammonium in the rumen, which is absorbed and transformed into urea in the liver. This increases its concentration in both blood and milk, and its excretion in the urine. These concentrations are important since they can have repercussions for the health, fertility or efficiency in the milk production of the animal (Noro & Wittwer 2003).

We observed a relationship (P <0.001) between the stability in the order of entrance to the milking parlour and milk fat contents (Table 5). There were no studies found on the association that might exist between the stability in the order of entrance to milking facilities and milk composition. It is known that breed, parity and stage of lactation affect milk yield and milk composition (Kelsey et al. 2003). In this study, we were not able to select animals based on parity or number of calvings, which could be helpful to explain the observed relationship.

In relation to the stability in the order of entrance to the milking parlour, the stable animals entered 1.7 positions after non stable animals, which agree with Hopster and van der Werf (1998) who found that cows with a consistent side choice took more time to enter the room. Rathore (1982) found that high-yield cows voluntarily entered the milking parlour earlier than low-yielding cows, and that was explained by the fact that cows were relieving the pressure of the udder caused by the milk, and that was the source of motivation to enter the milking parlour early. However, in the current study, we did not observed a relationship between the stability in the order of entrance to the milking parlour and milk yield. Conversely, Grasso et al. (2007) observed a positive correlation between milking order and milk production in primiparous cows, although the correlation coefficient was not high (r = 0.22). As shown in Table 5, there were no interactions between lactation stage (G) and stability (S) for milk yield, which could indicate that stability is a characteristic associated with the animal independently from its days in milk.

Overall, results showed that a cow that prefers to come in first place would likely have less variation in her milking position than a cow in the middle of the herd because it may be easier to consistently be first than consistently be last. Also, because there is less scope for variation at each end of the milking order. Although, our study used 205 animals, the heteroscedastic nature of milking position variance in the milking order was also reported in larger herds of around 500 lactating cows (Beggs et al., 2018).

Limitations of the study

Additional factors need to be taken into account when interpreting the data from this study. The weak correlations obtained are from the total available animals, without differentiating them by production level. Thus, there was a high percentage of the variance that is a consequence of other factors, some of which were considered in the analysis (lactation group and body weight) and others that the study did not account, such as the number of calvings and months of gestation. In this sense, the significance of the correlations could indicate that, by decreasing the sources of variation in the analyzed variables, the value of their correlation with social dominance could increase. Thus, although correlation values weak, they could be considered as the basis for a larger controlled experimental design in which the effect of the aforementioned variables are included or isolated.

Conclusion

In the current study, social rank was weakly correlated to production parameters such as milk yield, protein content, urea content, parity and body weight. Social rank did not affect cow's stability in the order of entrance to the waiting area and milking parlour. Stable cows had higher milk production and entered the milking parlour after non-stable animals. Overall, under the conditions of this study, the stability of the milking order appeared to be more closely associated with production traits rather than social rank.

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Table 1. Productive parameters, health related characteristics and social ranks (mean ± standard error)

Parameter	Group 1 (n = 77)	Group 2 (n = 63)	Group 3 (n = 65)
	0 – 100 days in milk	101 – 200 days in milk	>200 days in milk
Productive characteristics			
Milk yield, kg/d	31.6 ± 0.69	34.6 ± 0.52	34.0 ± 0.62
Fat, g/kg	37.5 ± 0.70	36.8 ± 0.80	34.8 ± 0.60
Protein, g/kg	32.2 ± 0.20	32.7 ± 0.40	32.9 ± 0.30
Milk urea N, mg/L	346 ± 9.6	366 ± 6.1	387 ± 5.6
Somatic cell counts, × 10^3 mL	210 ± 44.6	198 ± 30.1	207 ± 38.3
Live body weight, kg	622 ± 12.8	662 ± 12.0	700 ± 9.4
Number of lactations	2.06 ± 0.170	2.39 ± 0.170	1.86 ± 0.150
Health related characteristics			
Locomotion score	2.17 ± 0.050	2.08 ± 0.040	2.12 ± 0.040
Body condition score	3.07 ± 0.030	2.95 ± 0.020	3.10 ± 0.020
Udder hygiene score	1.65 ± 0.090	1.87 ± 0.080	2.03 ± 0.860

Table 2. Agonistic interactions recorded for 5 consecutive days in 205 dairy cows

Agonistic interactions	Group 1 (n = 77)	Group 2 (n = 63)	Group 3 (n = 65)
At the water troughs	232	178	147
At the feed bunks	800	630	685
Total interactions	1032	808	832
Social rank, number of cows			
Dominant	21	21	20
Intermediate	29	18	25
Subordinate	27	24	20

Table 3. Correlations between different parameters for animals with at least 5 encounters

Parameters	Correlation (R)	*P*-value
Parity and locomotion score	0.464	<0.001
Milk yield and body weight	0.445	<0.001
Milk yield and somatic cell count	- 0.101	0.308
Body condition score and locomotion score	- 0.016	0.824
Social rank and parity	0.339	<0.001
Social rank and milk protein content	0.293	<0.001
Social rank and body weight	0.276	<0.001
Social rank and milk urea content	0.242	<0.001
Social rank and milk yield	0.191	<0.001
Social rank and body condition score	0.126	0.095
Social rank and udder hygiene score	0.096	0.083
Social rank and locomotion score	0.056	0.518
Social rank and somatic cell count	-0.023	0.738
Social rank and milk fat content	- 0.126	0.056

Correlations were analysed using Pearson (parametric data: social rank, milk composition and milk yield) or Spearman (non-parametric data: locomotion score, body condition score, udder hygiene and dominance level) tests.

Table 4. Distribution of locomotion score (LS), body condition score (BCS) and udder hygine score (UHS) of lactating cows in relation to their social rank and Chi-square analysis

	Social rank							
Items	Dominant		Intermediate		Subordinate		All herd	
	n	%	n	%	n	%	n	%
LS								
LS 1	0	0	1	1	0	0	1	0.5
LS 2	54	89	63	84	62	90	179	88
LS 3	7	11	10	13	7	10	24	11
LS 4	0	0	1	1	0	0	1	0.5
LS 5	0	0	0	0	0	0	0	0
Cows $_{total}$	61		75		69		205	
BCS								
BCS 2.50	1	2	1	1	2	4	4	3
BCS 2.75	13	21	18	24	16	22	47	22
BCS 3.00	22	35	34	45	33	45	89	42
BCS 3.25	13	23	14	19	16	26	43	22
BCS 3.50	9	15	8	11	2	3	19	9
BCS 3.75	3	5	0	0	0	0	3	2
Cows $_{total}$	61		75		69		205	
UHS								
UHS 1	17	29	32	43	26	37	75	37
UHS 2	28	45	27	36	28	41	83	41
UHS 3	15	24	16	21	14	19	45	21
UHS 4	1	2	0	0	1	3	2	1
Cows $_{total}$	61		75		69		205	

LS	Dominant	Intermediate	Subordinate	X^2		
LS 1	0	1	0		Chi-square	3.94
LS 2	54	63	62		Degrees of freedom	6
LS 3	7	10	7	0.68	*P*-value	0.689
LS 4	0	1	0			
LS 5	0	0	0			

BCS	Dominant	Intermediate	Subordinate	X^2		
BCS 2.50	1	1	2		Chi-square	14.3
BCS 2.75	13	18	16		Degrees of freedom	10
BCS 3.00	22	34	33	0.16	*P*-value	0.155
BCS 3.25	13	14	16			
BCS 3.50	9	8	2			
BCS 3.75	3	0	0			

UHS	Dominant	Intermediate	Subordinate	X^2		
UHS 1	17	32	26		Chi-square	4.3
UHS 2	28	27	28	0.63	Degrees of freedom	6
UHS 3	15	16	14		*P*-value	0.634
UHS 4	1	0	1			

Table 5. Productive characteristics, social rank, and average order of entrance to the milking parlour from stable and no stable cows (mean ± standard deviation)

Parameters	Stable cows	No stable cows	*P*-value (G)	*P*-value (S)	*P*-value (G×S)
Waiting area					
Milk yield, kg/d	34.1± 0.67	32.3 ± 0.73	0.008	0.036	0.316
Fat, g/kg	36.0 ± 0.90	37.0 ± 0.70	0.155	0.362	0.818
Protein, g/kg	32.0 ± 0.30	32.0 ± 0.30	0.223	0.328	0.837
Milk urea N, mg/L	378 ± 6.7	351 ± 8.8	0.007	0.005	0.293
Somatic cell count, × 10^3/ml	169 ± 34.9	186 ± 33.4	0.291	0.994	0.396
Social rank	0.5 ± 0.02	0.5 ± 0.02	0.786	0.203	0.210
Order of entrance[1]	4.6 ± 0.28	4.7 ± 0.16	<0.001	0.637	0.799
Milking parlour					
Milk yield, kg/d	34.6 ± 0.61	33.7 ± 0.68	0.079	0.283	0.312
Fat, g/kg	36.5± 0.80	37.1 ± 0.70	0.489	<0.001	0.622
Protein, g/kg	32.4± 0.30	32.0 ± 0.30	0.153	0.491	0.860
Milk urea N, mg/L	371 ± 7.4	365 ± 8.5	0.005	0.618	0.569
Somatic cell count, × 10^3/ml	190 ± 37.9	202 ± 33.7	0.423	0.928	0.065
Social rank	0.5 ± 0.22	0.5 ± 0.24	0.703	0.619	0.644
Order of entrance[1]	6.9 ± 0.37	5.2 ± 0.18	<0.001	<0.001	<0.001

G = probability of lactation stage effect; S = probability of stability effect.
[1]Based on the different numbers of animals from Groups 1, 2 and 3, the positions in the order of entrance to the waiting area and milking parlour were standardize from 1 to 9.

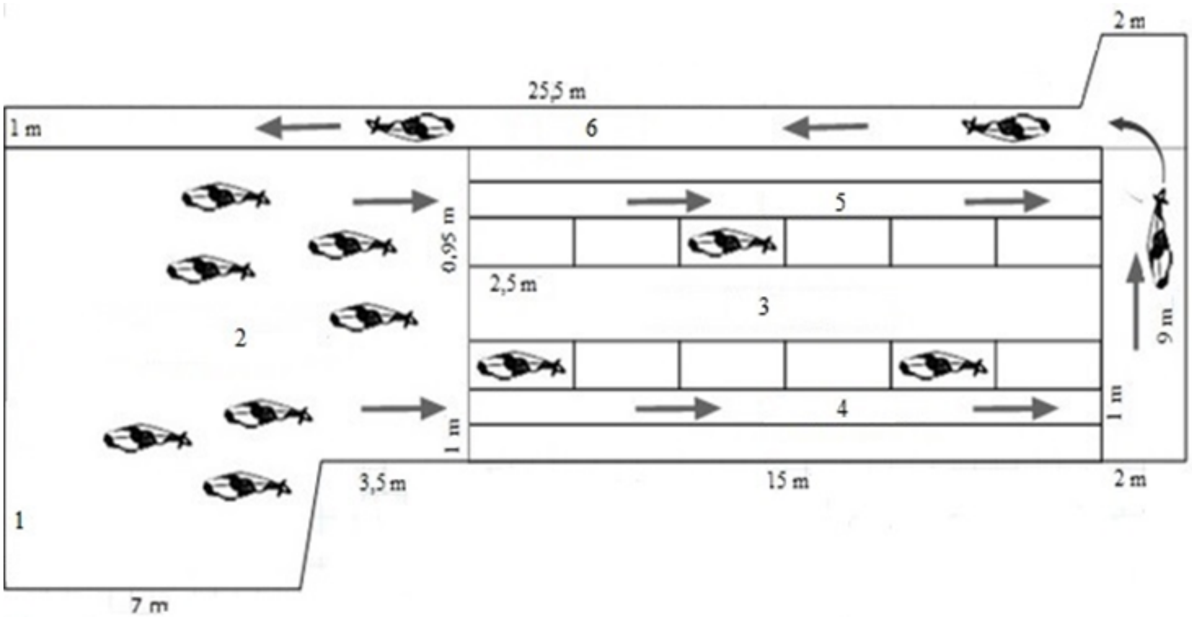
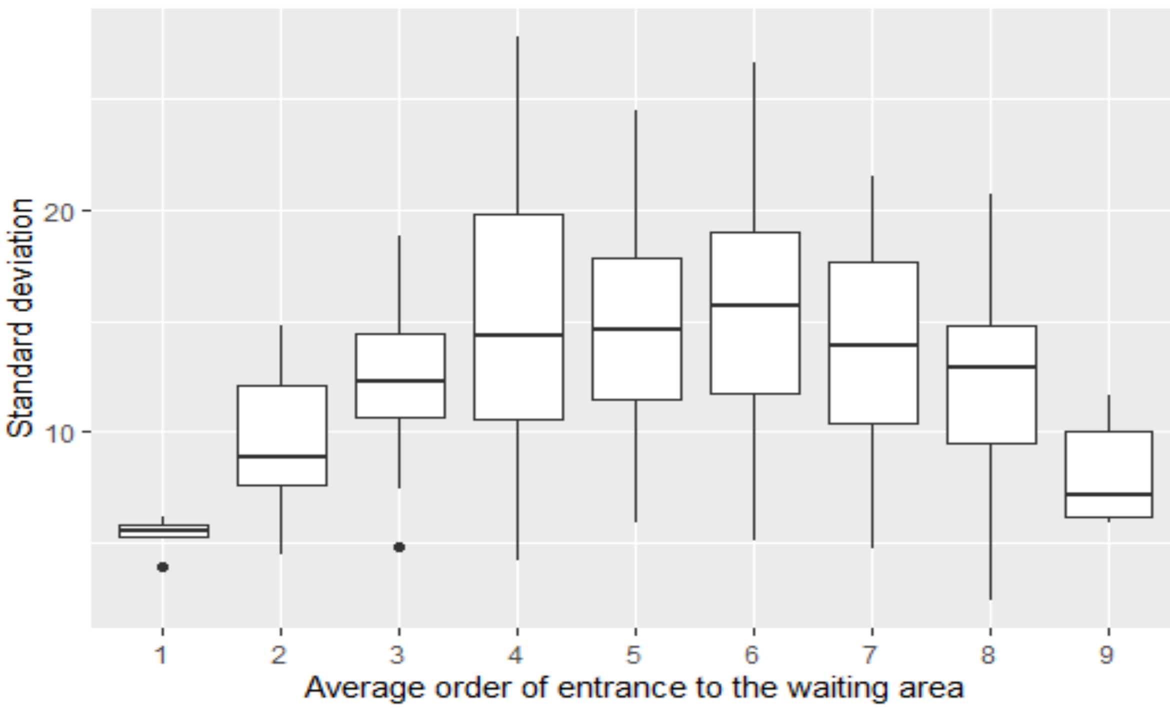


Figure 1. Layout of the 2 × 6 parallel milking parlour. 1-entrance, 2-waiting area, 3-operator´s pit, 4-right alley, 5-left alley, 6-exit alley.



Standard deviation
20
10
1
2
3
4
5
6
7
8
9
Average order of entrance to the waiting area

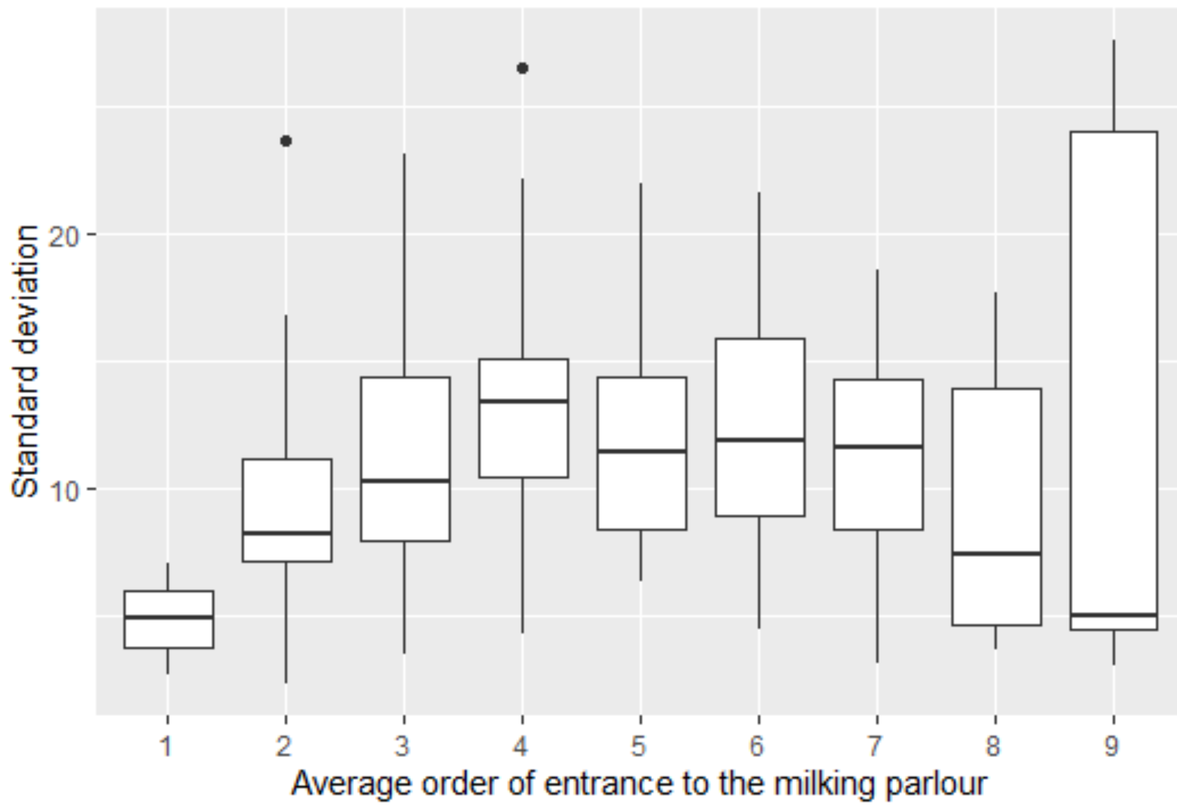


Figure 2. Notched Box Plots from average order of entrance to the waiting area and milking parlour